Journal of Surgical Oncology 64:262–267 (1997)

Roles of the Transforming Growth Factor β1 and Its Type I and II Receptors in the Development of a Pulmonary Adenocarcinoma: Results of an Immunohistochemical Study

IWAO TAKANAMI, MD,[1]* FUMIHIKO TANAKA, MD,[2] TOSHINORI HASHIZUME, MD,[3] AND SUSUMU KODAIRA, MD[1]

[1]*First Department of Surgery, Taikyo University School of Medicine, Tokyo, Japan*
[2]*Department of Pathology, Teikyo University School of Medicine, Tokyo, Japan*
[3]*Department of Surgery, National Sanatorium, Kanagawa Hospital, Kanagawa, Japan*

Background: In the United States, pulmonary adenocarcinomas have recently replaced squamous cell carcinomas as the most frequent type of lung cancer encountered. The incidence of pulmonary adenocarcinoma continued to increase worldwide.
Method: To determine the roles of the transforming growth factor-β1 (TGF-β1), and TGF-β type I receptor (TβR-I), and the TGF-β type II receptor (TβR-II) in the progression of a pulmonary adenocarcinoma, their respective expressions have been immunohistologically studied in specimens from 120 pulmonary adenocarcinoma patients.
Result: The overall prognosis was significantly poorer for patients showing positive TGF-β1, TβR-I, TβR-II expressions than for patients who were negative to all three immunostainings ($P < 0.01$). Our multivariate analysis also revealed that a positive TGF-β1 response significantly affect prognosis ($P < 0.05$).
Conclusions: TGF-β1, TβR-I, and TβR-II play important roles in tumor progression, and a positive TGF-β1 expression can serve as a pulmonary adenocarcinoma marker. TβR-I and TβR-II expressions are necessary for TGF-β signal transduction.
J. Surg. Oncol. 64:262–267, 1997 © 1997 Wiley-Liss, Inc.

KEY WORDS: **prognostic factor; proliferation; signal transduction; tumor progression**

INTRODUCTION

The transforming growth factor (TGF)-β constitutes a family of polypeptides that have been found to be multifunctional regulators for such processes as cell growth, differentiation, adhesion, migration, angiogenesis, extramatrical formation, and the immune functions [1,2]. Although the expression of TGF-β isoforms is differentially regulated, these pleiotrophic peptides have shown

*Correspondence to: Iwao Takanami, The First Department of Surgery, Teikyo University of Medicine, 11-1, Kaga 2-Chome, Itabashi-Ku, Tokyo, 173, Japan.
Accepted for publication 4 January 1996.

© 1997 Wiley-Liss, Inc.

similar biological effects in most experimental applications. As for cancer cell growth, the TGF-β peptides exert either a positive or a negative effect, depending on the cell type and culturing conditions. In this regard, they were found to suppress the proliferation of cancer cells in vitro [3]. As for cancer cell growth in vivo, however, failure to respond to the inhibitory activity of the TGF-β peptides has been speculated to confer a growth advantage to cancer cells [1,2].

Of the five different peptides that constitute the TGF-β family, TGF-β1 is the predominant form in humans, and it is widely distributed in a variety of normal cells and organ tissue [4]. Further, it has been found that the TGF-β activity is mediated through surface receptors to which the TGF-β peptides bind [5], and the TGF-β type I receptor (TβR-I), and the TGF-β type II receptor (TβR-II) possess intracellular serine/threonine kinase domains that are activated upon complex formation [6]. Further, TβR-III, membrane proteoglycan, binds TGF-βs but is not thought to have direct signal transducing activity [7].

The incidence of lung cancers of all histological types have increased, and in the United States, adenocarcinomas have recently replaced squamous cell carcinomas as the most frequent type of lung cancer encountered [8]. It also has been reported that the incidence of pulmonary adenocarcinomas is increasing globally [9]. Little progress has been made in what we know about pulmonary adenocarcinomas. Therefore, to add to our knowledge, this study has evaluated whether the TGF-β1, TβR-I, and TβR-II expressions play a role in the progression and prognosis of pulmonary adenocarcinoma.

MATERIALS AND METHODS

Studied were tissue specimens from 120 patients who underwent a pulmonary adenocarcinoma resection at the First Department of Surgery of Teikyo University between 1984 and 1991. Excluded were patients who died within 1 month after surgery and those who underwent exploratory thoracotomy. Also excluded were patients with a past history of another cancer.

The lesions of these 120 patients were staged on both the operative and pathologic findings, based on the International Union Against Cancer (UICC) TNM classification of 1987. Results broke down as follows: stage I in 58 patients; stage II in 6; stage IIIa in 32; stage IIIb in 2; and stage IV in 22. The patients consisted of 69 males and 51 females from 28–81 years (mean: 61 years).

Although the degree of histological differentiation in each pulmonary adenocarcinoma was evaluated, the degree of differentiation of an adenocarcinoma sometimes differed among areas of the same tumor, so that the most predominant degree of differentiation in each tumor was the deciding factor. On this basis, the lesions were found to be well differentiated in 57 patients, moderately differentiated in 44, and poorly differentiated in 19. Patients for whom radical surgery such as lobectomy or a pneumonectomy, with a hilar and mediastinal lymph node dissection, had been preoperative planned were considered to have manifested operative indications. Postoperatively, all patients were followed for 5–12 years and their outcomes were known.

Immunohistological Staining

Resected tissue specimens were fixed in formalin, embedded in paraffin, and cut into 3-μm serial sections. Then, using the rabbit anti-TGF-β1, TβR-I, and TβR-II polyclonal antibodies (TGF-β1: cat#sc-146; TβR-I: cat#sc-90) (Santa Cruz Biotechnology, Santa Cruz, CA) (TβR-II: #06-227) (Upstate Biotechnology, Lake Placid, NY), the sections underwent hematoxylin-eosin and immunohistological staining for TGF-β1, TβR-I, and TβR-II. The TGF-β1, TβR-I, and TβR-II antisera exhibited no cross reactivity on Western blotting or immunoprecipitation.

Immunohistological staining for TGF-β1, TβR-I, and TβR-II was based on the avidin biotin peroxidase complex (ABC) method and was performed by using a Vestatin Kit (Vector Co., Burlingame, CA). Briefly, the sections were deparaffinized and after inhibition of the endogenous peroxidase, were washed in phosphate-buffered saline (PBS). Next, the sections were treated with 10% normal swine serum (Vector), reacted with 1/50 solution of rabbit anti-TGF-β1, anti-TβR-I, and anti-TβR-II polyclonal antibodies as the primary antibodies, and stored at 4°C overnight. The secondary reaction was accomplished at room temperature by using biotinated swine anti-rabbit serum (Vector) for 60 minutes. Procedurally, the avidin biotin peroxidase was dripped onto the sections, after which the sections were not disturbed for 60 minutes. Then, the TGF-β1, TβR-I, and TβR-II were stained by diaminobenzidine. Nuclear staining was performed by using methyl green. Negative control sections were treated by using non-immunized rabbit IgG as the primary antibody.

Analysis

Two independent observers evaluated the immunohistological staining for TGF-β1, TβR-I, and TβR-II under light microscopy, and their expressions were classified as either negative or positive. Relationships among the TGF-β1, TβR-I, and TβR-II stainings were analyzed by using Spearman's correlation coefficients. The TGF-β1, TβR-I, and TβR-II stainings and relationships with the T-, N-, and M-factors, as well as the stage and the degree of histological differentiation, were analyzed by using the Chi-square test.

The survival rate was calculated by the Kaplan-Meier method and compared with log-rank test. Each prognostic factor was then correlated with overall survival in a multivariate analysis by using Cox's proportional hazard

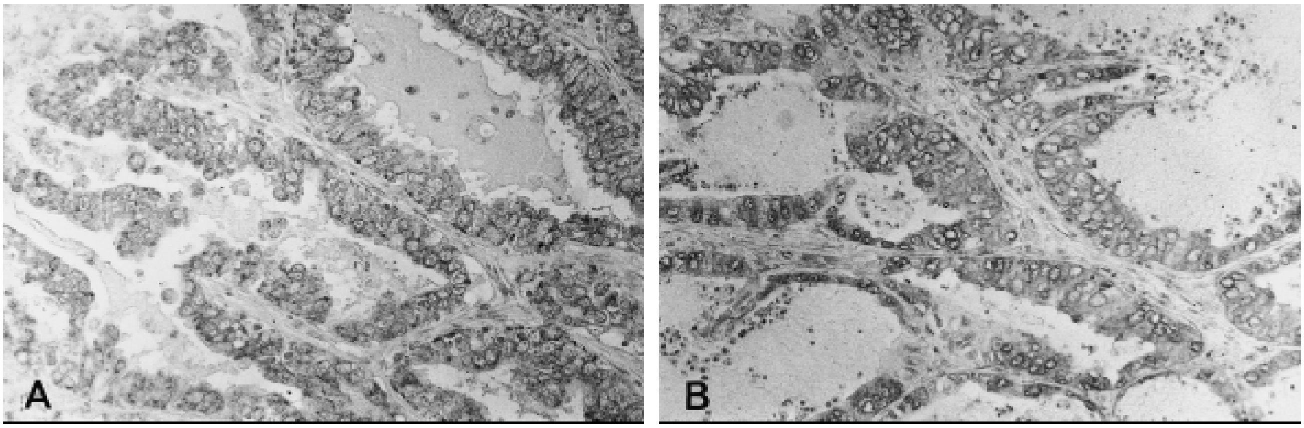

Fig. 1. A well-differentiated pulmonary adenocarcinoma (**A** and **B**). Most of the cytoplasms of the tumour cells showed intense TGF-β1 and TβR-I expressions. ×66 (A: TGFβ1 immunoreactivity; B: TβR-I immunoreactivity).

regression model. Computer calculations were performed by using the StatView statistical package (Abacus Concepts, Berkeley, CA) and a Macintosh System Power Book 5300 C computer. Variations that were statistically significant were set at $P < 0.05$.

RESULTS

The TGF-β1, TβR-I, and TβR-II immunohistological stainings were found to be slightly positive in the fibroblasts, the normal pulmonary alveoli, the bronchial epithelium, and the vascular endothelium. Further, in the cancer cells of many patients, the cytoplasms showed intense TGF-β1 and TβR-I stainings (Fig. 1A and B), and cancerous stroma showed intense TβR-II stainings (Fig. 2). Positive TGF-β1 and TβR-I stainings also were seen in the cancerous stroma and fibroblasts of the fibrous stroma of a small number of patients.

Of the 120 patients, positive TGF-β1, TβR-I, and TβR-II stainings were separately expressed in 66 patients (55%), 67 patients (56%), and 76 patients (63%), respectively. A significant correlation was found between TGF-β1 and TβR-I, but not among TGF-β1 and TβR-I, and TβR-II (Table I).

Table II shows the relationships among the TGF-β1, TβR-I, and TβR-II immunoreactivities and the clinicopathologic factors in our pulmonary adenocarcinoma patients. A significant relationship was found between the T-factor of the TNM classification and the TGF-β1 or TβR-I expression ($P < 0.01$ or $P < 0.05$). Further, a significant relationship was also found between the P-stage, the presence of metastases (M factor), and the TβR-II expression ($P < 0.05$ and $P < 0.01$).

Table III shows the relationship between the clinicopathologic features and the TGF-β1, TβR-I, and TβR-II expressions on comparing the findings between the all negative group and the all positive group. This comparison revealed significant differences in the *P* stage ($P <$

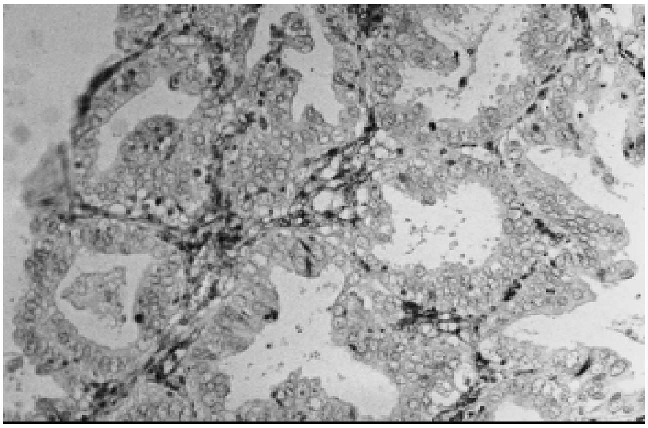

Fig. 2. A well-differentiated pulmonary adenocarcinoma. The mesenchymal portion of the tumour cells showed a positive TβR-II expression. ×66

TABLE I. Pulmonary Adenocarcinoma: The Spearman TGF-β1, TβR-I, and TβR-II Correlation Coefficients

	Parameters		
	TGF-β1	TβR-I	TβR-II
TGF-β1	1		
TβR-I	0.65	1	
TβR-II	0.07	0.06	1

0.01) and the M factor ($P < 0.05$) between these two groups.

Figures 3, 4, and 5, graphically show the overall prognosis, based on the TGF-β1, TβR-I, and TβR-II classification. Significant differences were seen between the TGF-β1 (−) and the TGF-β1 (+) survivals ($P < 0.01$), and between the TβR-II (−) and the TβR-II (+) survivals ($P < 0.05$).

Table IV shows the 5-year survival rate in patients whose tumors showed positive TGF-β1, TβR-I, and TβR-II immunostainings versus patients with tumors that were negative to all three immunostainings. This log-

TABLE II. Relationships Among TGF-β1, TβR-I, and TβR-II Immunoreactivities and Clinicopathologic Factors in Pulmonary Adenocarcinoma Patients

Variable	No. of cases	No. of cases with immunoreactivity to: TGF-β1	TβR-I	TβR-II
P-stage[a]				
I	58	27 (46.6%)	26 (44.8%)	30 (51.7%)
II	6	3 (50.0%)	3 (50.0%)	3 (50.0%)
III a	32	18 (56.3%)	22 (68.8%)	22 (68.8%)
III b	2	2 (100.0%)	2 (100.0%)	2 (100.0%)
IV	22	16 (72.7%)	14 (63.6%)	19 (86.4%)
P value[b]		NS	NS	$P < 0.05$
T factor[a]				
T1	51	20 (39.2%)	23 (45.1%)	32 (62.7%)
T2	60	39 (65.0%)	36 (60.0%)	39 (65.0%)
T3	9	7 (77.8%)	8 (88.9%)	5 (55.6%)
P value[b]		$P < 0.01$	$P < 0.05$	NS
N factor[a]				
NO	69	35 (50.7%)	35 (50.7%)	38 (56.3%)
N1	6	3 (50.0%)	3 (50.0%)	3 (50.0%)
N2	41	25 (61.0%)	26 (63.4%)	32 (78.0%)
N3	4	3 (75.0%)	3 (75.0%)	3 (75.0%)
P value[b]		NS	NS	NS
M factor[a]				
MO	98	50 (51.0%)	53 (54.1%)	57 (58.8%)
M1	22	16 (72.7%)	14 (63.6%)	19 (86.3%)
P value[b]		NS	NS	$P < 0.01$
Differentiation				
Well	57	31 (54.3%)	31 (54.4%)	34 (59.6%)
Moderate	43	27 (62.8%)	28 (65.1%)	32 (74.4%)
Poor	20	8 (40.0%)	8 (40.0%)	10 (50.0%)
P value[b]		NS	NS	NS

[a]TNM lung cancer staging system of the International Union Against Cancer (UICC).
[b]Chi-square test.
NS = not significant.

rank analysis indicates that patients whose tumors stained positive for TGF-β1, TβR-I, and TβR-II had a significantly poorer prognosis than patients whose tumors were negative to all three immunostainings ($P = 0.0013$).

To determine whether the TGF-β1, TβR-I, and TβR-II expressions could serve as prognostic indicators of postoperative overall survival, a multivariate analysis was done on specimens from 96 potentially curatively operated patients (Table V). The results of this analysis revealed that the TGF-β1 expression ($P = 0.0188$) and the *P*-stage ($P = 0.0001$) can serve as prognostic indicators of postoperative overall survival.

DISCUSSION

Various human cancers express the TGF-β polypeptides. Elevated TGF-β1 levels have been reported in gastric [10], thyroid [11], and brain cancers [12], and TGF-β1 expression has been found to relate the progression of breast cancer [13]. As for the mechanisms accounting for this TGF-β expression, it is speculated that the TGF-β signal is transduced through two receptors, TβR-I and TβR-II that function as a complex.

Further, as we have previously reported, the TGF-β1

TABLE III. Pulmonary Adenocarcinoma: Relationships Between Clinicopathologic Features and TGF-β1, TβR-I, and TβR-II Expressions on Comparing Findings Between the All-negative Group and the All-positive Group*

	TGF-β1 (−) TβR-I (−) TβR-II (−)	TGF-β1 (+) TβR-I (+) TβR-II (+)	*P*-value[a]
Patient[b]	19	36	
P-stage			
I	14	10	
II	2	2	$P < 0.01$
IIIa	2	10	
IIIb	0	2	
IV	1	12	
T factor[b]			
T1	11	12	
T2	8	20	NS
T3	0	4	
N factor[b]			
N0	14	15	
N1	2	2	NS
N2	2	16	
N3	1	3	
M factor[b]			
M0	18	24	
M1	1	12	$P < 0.05$
Differentiation			
Well	10	16	
Moderate	4	17	NS
Poor	5	3	

*TGF = transforming growth factor; TβR = transforming growth factor-β receptor.
[a]Chi-square test.
[b]TNM Lung Cancer staging system of the International Union Cancer (UICC).

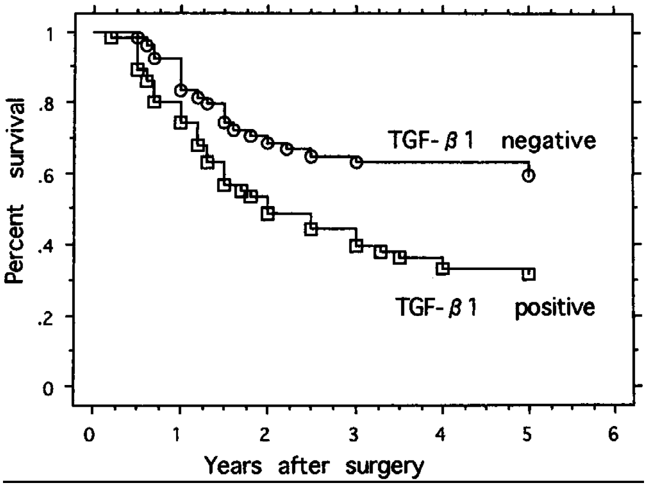

Fig. 3. Overall survival curves of the pulmonary adenocarcinoma cases, based on the TGF-β1 response. A significant difference was seen between the negative and positive cases ($P < 0.01$).

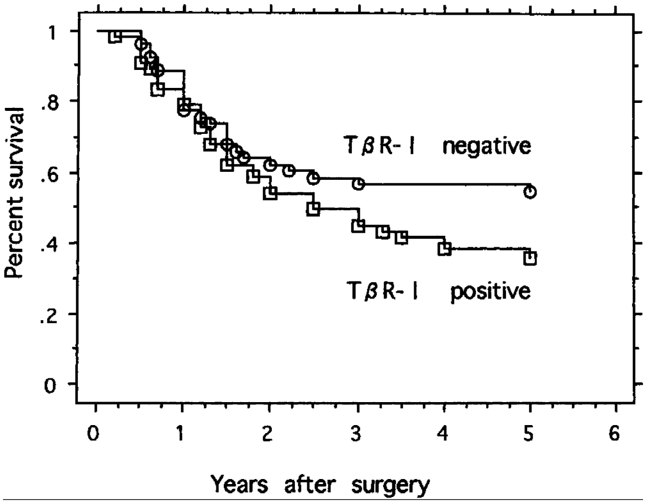

Fig. 4. Overall survival curves of the pulmonary adenocarcinoma cases, based on the TβR-I response. A significant difference was not seen between the negative and positive cases.

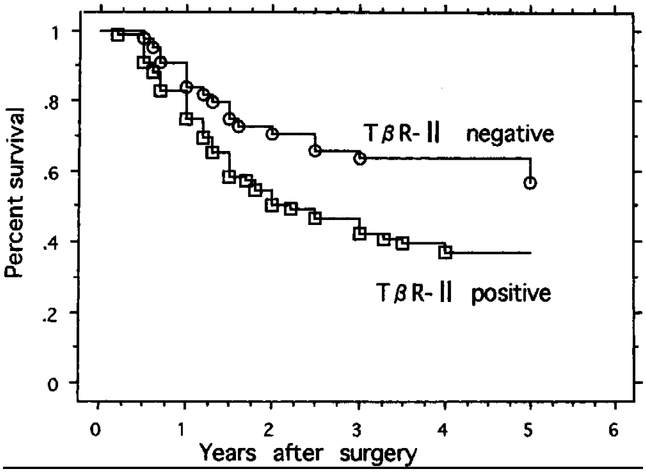

Fig. 5. Overall survival curves of the pulmonary adenocarcinoma cases, based on the TβR-II response. A significant difference was seen between the negative and positive cases ($P < 0.05$).

expression was found to be a prognostic factor in pulmonary adenocarcinomas [14]. Also, in pancreatic cancers, it has been found that the TGF-β1 and TβR-II mRNA levels are increased [15,16]. However, until this study, the frequency and correlations of the TGF-β1, TβR-I and TβR-II expressions in pulmonary adenocarcinomas have not been investigated.

Immunohistochemical techniques were used to evaluate TGF-β1, TβR-I, and TβR-II expressions in pulmonary adenocarcinomas, and on immunostaining the TGF-β1 and TβR-I responses were primarily cytoplasmic, whereas the TβR-II response was mesenchymal. These findings suggest that in pulmonary adenocarcinomas the TGF-β polypeptides may act in an autocrine and paracrine manner to activate the expression of TβR-I and TβR-II.

TABLE IV. Five-year Survival Rate in Lung Adenocarcinoma Patients Whose Tumors Showed Positive TGF-β1, TβR-I, and TβR-II Immunostainings vs. Patients With Tumors That Were Negative to All Three Immunostainings*

	n	5-year survival rate	Log-rank
TGF-β1 (−), TβR-I (−), TβR-II (−)	19	68%	-
TGF-β1 (−), TβR-I or TβR-II (+)	27	51%	0.2453
TGF-β1 (−), TβR-I (+), TβR-II (+)	8	63%	0.8360
TGF-β1 (+), TβR-I (−), TβR-II (−)	2	50%	0.7770
TGF-β1 (+), TβR-I or TβR-II (+)	16	43%	0.0582
TGF-β1 (+), TβR-I (+), TβR-II (+)	36	25%	0.0013

*TGF: transforming growth factor; TβR: transforming growth factor-β receptor.

TABLE V. Multivariate Analysis of 96 Curatively Resected Lung Adenocarcinoma Patients Using Cox's Proportional Hazard Model

	Multivariate analysis	
Variables	χ^2	*P* value
P stage	26.206	0.0001
TGF-β1	5.523	0.0188
TβR-I	1.448	0.2288
TβR-II	0.538	0.4634

TGF = transforming growth factor; TβR = transforming growth factor-β receptor.

In this regard, TGF-β1 was detected in patients who showed an intense TβR-I expression, and a significant correlation was found between the expression of TGF-β1 and TβR-I, but no correlation was found between either the TGF-β1 or TβR-I expression and the TβR-II expression. Similar findings have been reported in glioblastoma by Yamada et al. [17].

We also found that positive TGF-β1 and TβR-I expressions are associated with growth in tumor size. After reaching a certain size, the tumor acquires vascularization and TGF-β1 and TβR-I is speculated to be involved in this process. As for TβR-II expression, it showed an association with a worsening tumor stage and the M-factor. In glioma as well, the TβR-II expression has been reported to correlate with the grade of malignancy [17].

Our studies showed that less advanced tumor showed no response to TGF-β1, TβR-I, and TβR-II immunostainings, whereas more advanced tumors responded to all three immunostainings. These findings were similar, which probably reflects a close TβR-II involvement in developing malignancies. Further, the overall prognosis of patients who showed a positive TGF-β1 or TβR-II response was poorer than that of patients who showed negative TGF-β1 or TβR-II response, and the 5-year survival rate of patients whose tumor cells did not express TGF-β1, TβR-I, and TβR-II antibodies was markedly higher. Conversely, the 5-year survival rate of pa-

tients was significantly poorer in patients in whom the three antibodies were expressed in their tumor cells.

The results of our multivariate analysis revealed that the TGF-β1 expression has a significant effect on prognosis. Also, although the TβR-II expression appeared to be a prognostic indicator, our analysis revealed that the TβR-II expression is not an independent indicator, and it may be that the TβR-II expression interferes with the effect of stage on survival.

Our findings suggest that a pulmonary adenocarcinoma is more likely to proliferate and metastasize when TGF-β1, TβR-I, and TβR-II are expressed. In contrast, a reduced expression of TGF-β1, TβR-I, and TβR-II correlated with less tumor aggressiveness and a better prognosis. It has been suggested that the p53 [18], the retinoblastoma suppressor protein [19], and the c-myc proliferation-inducing protein [20] may be included in TGF-β mediated signal transduction pathways. Therefore, it is possible to speculate that the expression of TGF-β1, TβR-I, and TβR-II may confer a growth advantage to in vivo cancer cells.

Further, pulmonary adenocarcinomas have been found to overexpress the epidermoid growth factor (EGF) [21], EGF-receptor [21], c-erbB-2 [22], TGF-α [23], other growth factors and their receptors [24]. Thus it may be that the growth advantage derived by pulmonary adenocarcinomas that express TGF-β1, TβR-1, and TβR-II also may depend on the participation of these other regulatory signals.

Based on the above findings, it thus appears that TGF-β1, TβR-I, and TβR-II expressions play an important role in determining tumor progression and that the TGF-β1 value is a useful prognostic marker for a pulmonary adenocarcinoma. Further, the results of our studies on signal transduction mechanisms of TGF-β suggest that the presence of a TβR-I and TβR-II expression is required for TGF-β signal transduction.

CONCLUSION

The overall prognosis of patients who showed a positive TGF-β1 or TβR-II response was poorer than that of patients who showed a negative TGF-β1 or TβR-II response. The 5-year survival rate was significantly poorer for patients showing positive TGF-β1, TβR-I, TβR-II expressions than for patients in whom all three immunostainings were negative. These findings suggest that TGF-β1, TβR-I, and TβR-II play important roles in tumor progression and that positive TβR-I and TβR-II expression are necessary for TGF-β signal transduction.

REFERENCES

1. Massague J: The transforming growth factor-β family. Ann Rev Cell Biol 6:597–614,1990.
2. Sporn MB, Roberts AB: The transforming growth factor-β: Recent progress and new challenges. J Cell Biol 119:1017–1021,1992.
3. Sporn MB, Torando GJ: Autocrine secretion and malignant transformation of cells. N Engl J Med 303:878–880,1980.
4. Miyazono K, Hellman U, Wernstedt C, Heldine CH: Latent high molecular weight complex of transforming growth factor β1. J Biol Chem 263:6407–6415,1988.
5. Kingsley DM: The TGF-β superfamily: New members, new receptors, and new genetic tests of function in different organisms. Genes Dev 8:133–146,1994.
6. Wrana JL, Attisano L, Wieser R, et al.: Mechanism of activation of the TGF-β receptor. Nature 370:341–347,1994.
7. Lopex-Casillas F, Wrana JL, Massague J: Betaglycan presents ligand to the TGF-β signaling receptor. Cell 73:1435–1444,1993.
8. Travis WD, Travis LB, Devesas SS: Lung cancer. Cancer 75:191–202,1995.
9. Valaitis J, Warren S, Gamble D: Increasing incidence of adenocarcinoma of the lung. Cancer 47:1042–1047,1981.
10. Hirayama D, Fujimori T, Satonaka K, et al.: Immunohistochemical study of epidermal growth factor and transforming growth factor-β in the penetrating type of early gastric cancer. Hum Pathol 23:681–685,1992.
11. Jasani B, Wyllie FS, Wright PA, et al.: Immunohistochemically detectable TGF-β is associated with malignancy in thyroid epithelial neoplasia. Growth Factors 2;149–155,1990.
12. Johnson MD, Federspiel CF, Gold LI, Moses HL: Transforming growth factor-β and transforming growth factor β receptor expression in human meningioma cell. Am J Patho 141:633–642,1992.
13. Gorsch SM, Memoli VA, Stukel TA, et al.: Immunohistochemical staining for transforming growth factor β1 associates with disease progression human breast cancer. Cancer Res 52:6949–6952,1992.
14. Takanami I, Imamura T, Hashizume T, et al.: Transforming growth factor β1 as a prognostic factor in pulmonary adenocarcinoma. J Clin Pathol 47:1094–1100,1994.
15. Friess H, Yamanaka Y, Büchler M, et al.: Enhanced expression of transforming growth factor β isoforms in pancreatic cancer correlates with decreased survival. Gastroenterology 105:1846–1856,1993.
16. Friess H, Yamanaka Y, Büchler M, et al.: Enhanced expression of the type II transforming growth factor β receptor in human pancreatic cancer cells without alteration of type III receptor expression. Cancer Res 53:2104–2707,1993.
17. Yamada N, Kato M, Yamashita H, et al.: Enhanced expression of transforming growth factor-β (TGF-β) and its type I and type II receptors in human glioblastoma. Int J Cancer 62:386–392,1995.
18. Fults D, Brockmeyer D, Tullous MW, et al.: p53 mutation and loss of heterozygosity on chromosomes 17 and 10 during human astrocytoma progression. Cancer Res 52:674–679,1992.
19. Sherr CJ: The ins and out of RB: coupling gene expression to the cell-cycle clock. Trends Cell Biol 4:15–18, 1994.
20. Pietenpol JA, Munber K, Howley PM, et al.: Factor-binding element in the human c-myc promoter involved in transcriptional regulation by transforming growth factor β1 and by the retinoblastoma gene products. Proc Nat Acad Sci 88:10227–10231,1991.
21. Tateishi M, Ishida T, Mitsudomi T, et al.: Immunohistochemical evidence of autocrine growth factors in adenocarcinoma of the human lung. Cancer Res 50:7077–7080,1990.
22. Tateishi M, Ishida T, Mitsudomi T, et al.: Prognostic implication of transforming growth factor α in adenocarcinoma of the lung-an immunohistochemical study. Br J Cancer 63:130-133,1991.
23. Tateishi M, Ishida T, Mitsudomi T, et al.: Prognostic value of c-erbB-2 protein expression in human lung adenocarcinoma and squamous cell carcinoma. Eur J Cancer 27:1372–1375,1991.
24. Takanami I, Tanaka F, Hashizume T, et al.: Expression of basic fibroblast growth factor and its receptor are involved in the progression of pulmonary adenocarcinoma. Eur J Cancer 32A:1504–1509,1996.